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SHORT COMMUNICATION

Fine structure of the stinger (aculeus) in *Euscorpius*

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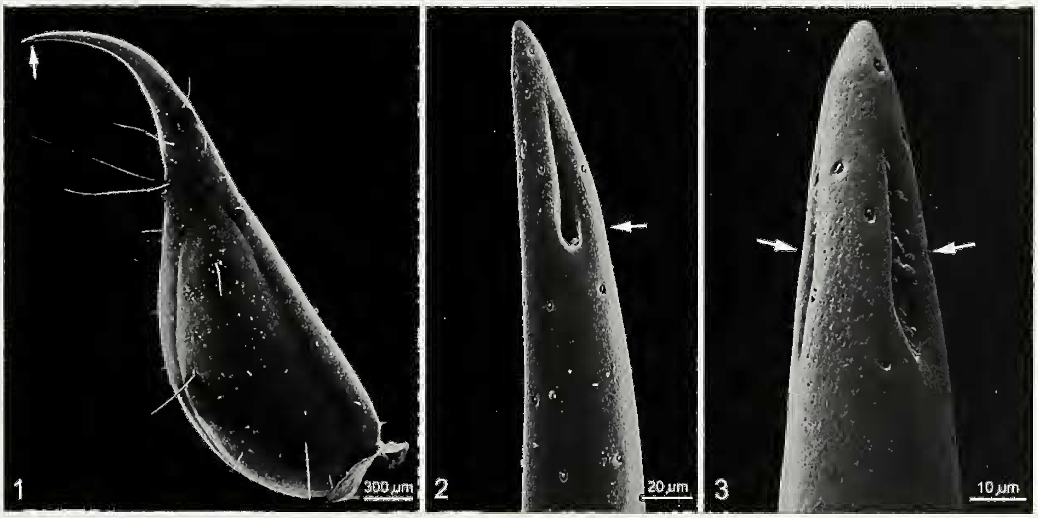
Abstract. A scorpion's last metasomal segment (telson) consists of a bulbous base that contains two venom glands and a curved tip (aculeus) where two venom ducts open to the outside. These two openings lie laterally just before the very tip of the aculeus; to see both of them at the same time, the stinger has to be looked at "tail-on" from the dorsal side. The two venom ducts have a distinct cuticular lining, which can be recognized in a transparent exuvia as long tubes (1 mm) extending from the distal pores back to the venom glands. Whereas the proximal bulb has many long sensory hairs on its surface, the distal aculeus is very smooth but contains small pits with tiny club-shaped hairs. These are probably contact chemoreceptors. The advantage of such sunken sensory hairs is certainly that the stinger can penetrate into prey (or foe) but can still perceive mechanical or chemical stimuli. Additionally, the aculeus bears several slit sensilla and numerous fine pores of unknown function. The aculeus is thus not only a well-adapted injection device but also contains sensory structures, which provide information on mechanical and chemical input.

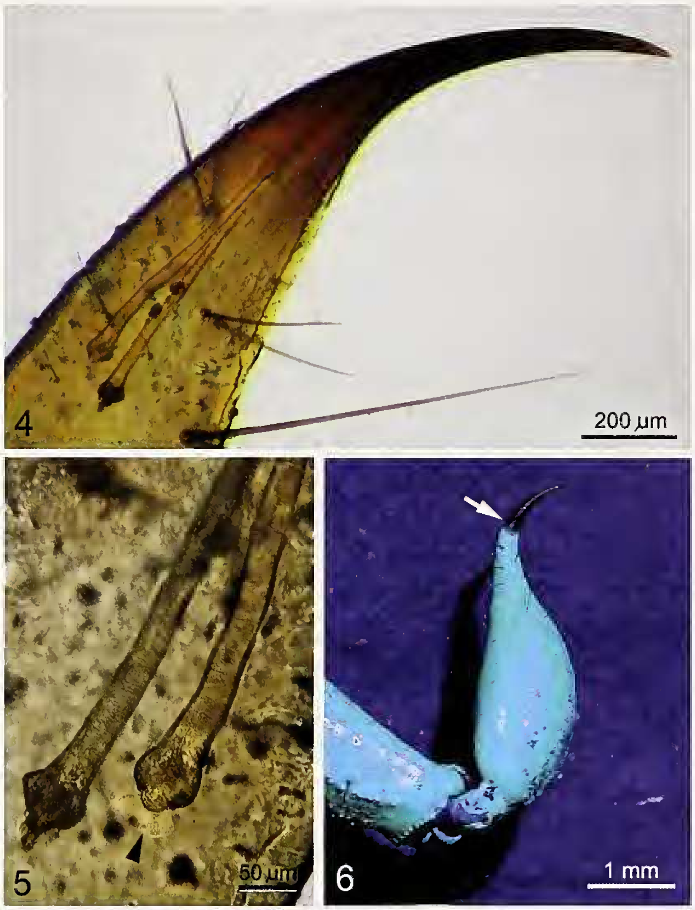
Keywords: Scorpions, stinger, aculeus, fine structure

It is common knowledge that a scorpion delivers its venomous sting with the tip of its tail (Hjelle 1990; Braunwalder 2005; Mahsberg et al. 2012). However, many arachnologists are unaware that there are in fact two small venom openings near the very tip. This is understandable because these pores are quite small (less than 10 µm in diameter) and hard to detect within the dark tip of the stinger. It is remarkable that some of the very early microscopists had already noted both openings. For instance, Antoni van Leeuwenhoek (1700) described in a letter to the Royal Society: "*... when we observe the sting through the magnifying glass, we find that the sting has an opening on either side close to the sharply pointed part ...*". A more detailed picture was given by Maupertuis (1733) by conducting a small experiment, namely squeezing the bulb of the stinger: "*Si l'on presse fortement la fiole, ...on voit la liqueur qu'elle contient, s' échapper à droite & à gauche, par ces deux trous.*" (If one presses the bulb strongly ... one can see the liquid that it contains, exiting on the left and right side, through two holes.") Later, Joyeux-Laffuie (1883) made histological sections of the stinger (telson) and provided illustrations of the tip (aculeus) in lateral and dorsal views. Modern microscopical techniques have rarely been used to examine a scorpion's stinger, perhaps with the exception of a SEM (scanning electron microscope) picture showing both venom openings with congealed venom oozing out (Farley 1999). We have looked at the stinger of several species of *Euscorpius* using light microscopy and SEM to illustrate the dual opening clearly, and to study the overall organization of the exterior and interior of the aculeus.

We used mostly exuviae from *Euscorpius flavicaudis* De Geer 1778, but also from the species *E. italicus* Herbst 1800, *E. germanus* Koch 1837, *E. alpha* Caporiacco 1950 *and E. tergestinus* Koch 1837. All specimens were from scorpions bred in captivity by one of the authors



Figures 1–3.—1, Lateral view of the last tail segment (telson) in *E. flavicaudis* showing a bulbous base proximally and the curved stinger (aculeus) distally. The venom exits near the tip of the aculeus (arrow). Several sensory hairs cover the bulb, but are lacking on the smooth aculeus. 2, Aculeus tip laterally (*E. flavicaudis*), showing a subterminal venom opening (arrow). A corresponding opening would be visible on the other side. 3, A dorsal view of the aculeus tip (*E. flavicaudis*) shows two elongated venom openings side by side (arrows). Note several small dimples, containing very short sensory hairs.



Figures 4–6.—4, A bleached stinger (*E. flavicaudis*) reveals a rather solid tip and two cuticular tubes inside, which originate from the two venom glands. 5, Higher magnification of the initial part of the venom ducts. A very delicate collar (arrowhead) makes the connection to the distal end of the venom glands lying inside the bulb of the telson. 6, UV illumination of the telson (*E. italicus*) causes a bright green fluorescence, except for the distal stinger, which remains black. Note the sharp borderline (arrow) between the fluorescent and the non-fluorescent part of the stinger. (Photo by Bastian Rast).

(MB). For light microscopy, stingers were bleached in lactic acid for several hours, washed, and then immersed in 70% alcohol for bright field, dark field and phase contrast examination. Photographs were taken with a digital camera (Canon 600D) attached to a Leitz Diaplan microscope. For scanning electron microscopy (SEM) stingers were dissected under 70% alcohol, then dehydrated in acetone, and after air-drying, carefully mounted and oriented on aluminum stubs. After sputter coating with gold they were examined in a Zeiss DSM 950; digital photographs were taken at 20–5000x.

The last tail segment, the telson, measures 5–6 mm in length in *Euscorpius* species. Its base is bulbous and contains the two venom glands; its distal end, the aculeus, is curved and narrows into a needle-like tip. Microscopic examination shows that the bulb is covered with sensory hairs, mostly on the ventral side. In contrast, the distal aculeus appears smooth and does not bear long sensory hairs (Fig. 1). However, at high magnification small dimples containing tiny club-shaped sensilla become visible (Figs. 2, 3); they are surrounded by numerous tiny pores (ca. 0.1 µm diameter; Figs. 3, 7, 9), which can only be discerned under the electron microscope. These nanopores occur only on the distal half of the aculeus and may be related to an equally restricted network of nanometer canals, which are involved in the deposition of heavy metals (Schofield et al. 2003; see below).

The most conspicuous features of the aculeus are the venom openings, which are located laterally, about 0.1 mm away from the very tip of the stinger. Normally, only one opening is visible, because the stinger is usually seen from the side (Fig. 2). Only if the telson is viewed from behind ("tail-on") can both venom openings be observed in one picture. Although this can be convincingly demonstrated with the SEM (Figs. 3, 7), it is almost impossible with the light microscope. Firstly, the diameter of these pores is only 6–7 µm, and secondly, the cuticle of the aculeus tip is rather dark and almost solid, thereby obscuring fine structural details. We were partly successful, however, if we used preparations that had been bleached for several hours in lactic acid. In those cases one can vaguely see the actual venom openings near the stinger's tip and, much more clearly, the two venom ducts inside the aculeus, leading back to venom glands (Figs. 4, 5). This distinct visibility is due to a cuticular lining of the venom ducts, which is preserved during ecdysis. These ducts are about 1 mm long and 30 µm in diameter; the wall thickness is about 1 µm. It appears that each duct is connected to the distal end of the venom gland by a fine cuticular collar (Fig. 5).

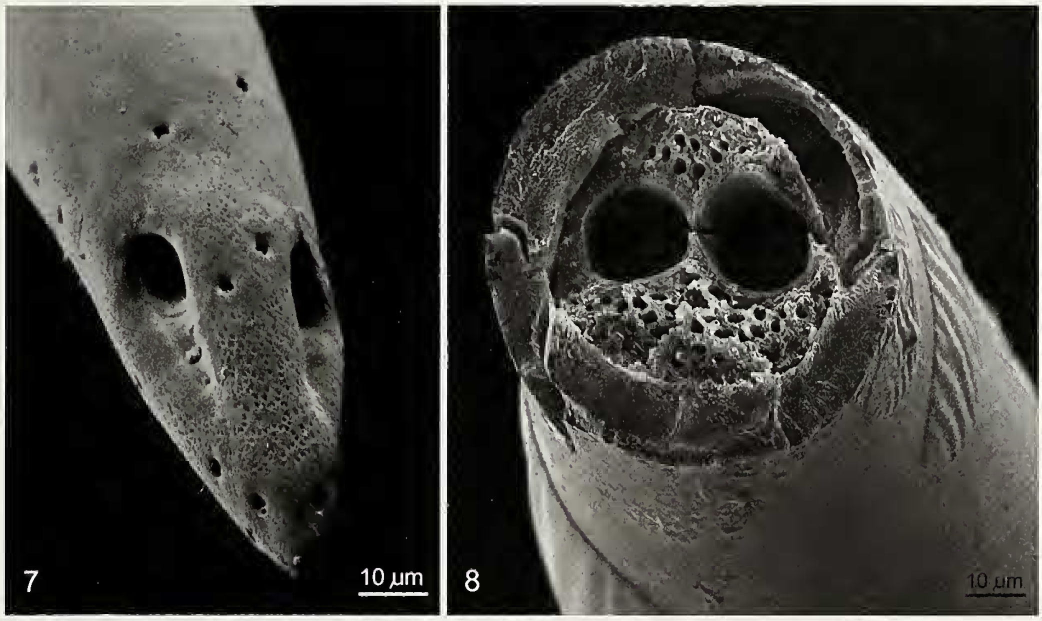
Because we did not know whether the two venom ducts would eventually fuse distally into one channel, we cut off the very end of the aculeus tip with fine scissors and looked at the cross-sectioned stinger under the SEM (Fig. 8). All our preparations exhibited two separate venom ducts, where the medial walls touched but never fused. Both tubes were embedded in a slightly porous cuticle, which was peripherally surrounded by the solid wall of the stinger. Thus, despite its seemingly delicate nature, the aculeus tip must be rather rigid and resistant to mechanical stress.

As mentioned above, the distal-most mm of the aculeus bears no projecting sensory hairs, but only tiny sunken sensilla and very small pores – both of which are not present on the proximal telson (bulb). However, we did observe a few larger pores that are most likely openings of dermal glands (Fig. 11). And we also found a few slit sensilla with their slits oriented perpendicular to the long axis of the aculeus (Fig. 13). A few short hair sensilla (40 µm long, 4 µm in diameter; Fig. 12) were detected in the proximal part of the aculeus. Based on their morphology (blunt tip, distinct socket), they could be contact chemoreceptors (Foelix & Schabronath 1983; Foelix 1985; Gaffin & Brownell 2001).

A remarkable property of scorpion cuticle is its bright green fluorescence in response to longwave ultraviolet (UV) illumination (Pavan 1954). Although this is true for the entire body cuticle, there is one exception: the distal (black) end of the aculeus does not fluoresce. Under daylight illumination the transition into the dark aculeus tip appears as a gradual change, but under UV light there is a sharp borderline between the proximal fluorescent telson and the completely black, non-fluorescent aculeus tip (Fig. 6). We assume that the cuticle of that distal region lacks the fluorescent substances (β-carboline and coumarin) that are normally present in the body cuticle (Stachel et al. 1999; Frost et al. 2001). Unfortunately, hardly anything is known about the possible biological significance of fluorescence in scorpion cuticle (Gaffin et al. 2012).

A conspicuous feature of the aculeus is the smooth surface of the last millimeter of its tip. This is certainly advantageous for an easy and relatively deep penetration into the prey's tissues. Any hair sensilla projecting from the surface are restricted to the proximal part of the telson. However, several tiny club-shaped sensilla do occur in the tip region, but they are hidden in tiny dimples. It is quite likely that they represent contact chemoreceptors. Very similar sunken sensilla were found on the jaws (maxillae) of ant lions, which are also used for venom injection into prey (R. Foelix unpublished results).

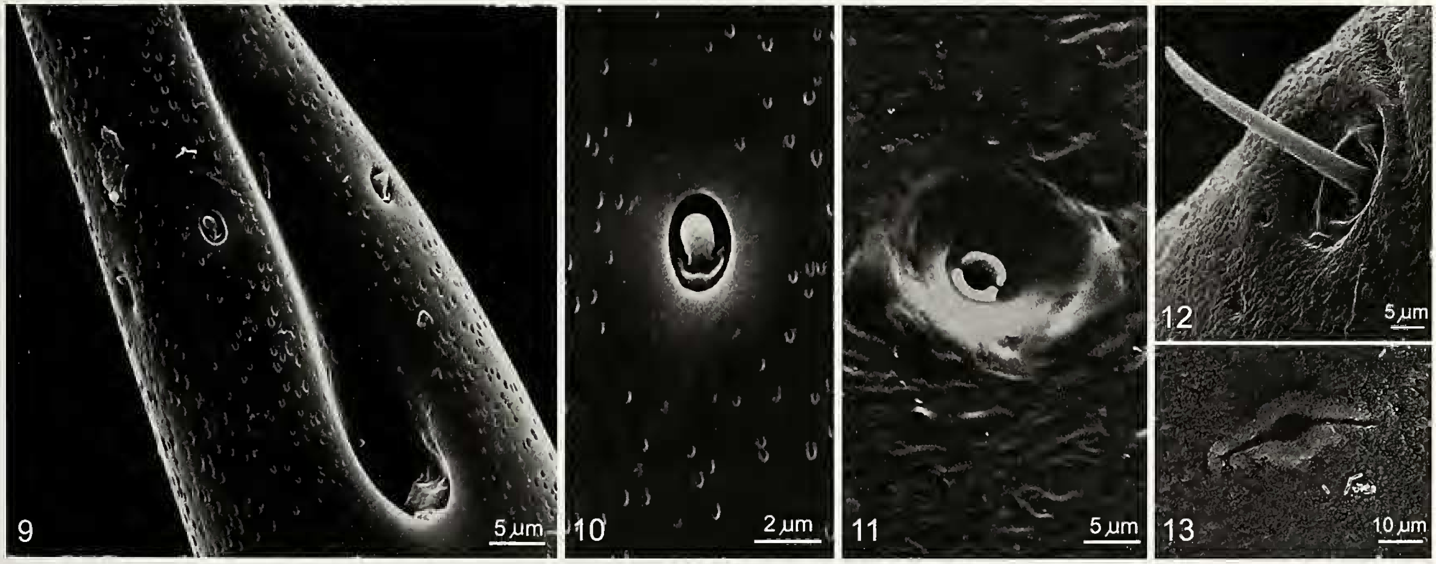
Additionally, there are several single slit sensilla embedded in the cuticle of the aculeus, which provide information on mechanical strain. The aculeus is thus not merely an injection device, but is also able to integrate mechanical and chemical stimuli. This is also indicated by the presence of a telson nerve running between the two venom ducts (Farley 1999).



Figures 7, 8.—7, A posterior view of the aculear tip (*E. flavicaudis*) showing the two venom openings and small dimples containing sunken sensory hairs. 8, If the very tip of the aculeus (*E. italicus*) is snipped off with scissors, the two venom ducts are seen in cross-section; they are surrounded by a porous cuticle inside and a solid cuticle wall in the periphery.

The function of the dermal glands on the telson is not really known, but it is possible that they play a role in courtship. During early courtship the male scorpion often stings the female into her joint membranes, leaving the aculeus there for up to 10 min (Francke 1979). It is not clear whether this implies an actual venom injection or a transfer of other chemical substances, which may originate from the aculear dermal glands. Another possibility is that these dermal glands produce a sex pheromone in females; it has been reported that female cuticle extracts induce courtship patterns (tail wagging and pedipalp reaching) in male scorpions (Gaffin & Brownell 1992).

The venom ducts are lined by a thin cuticle and are firmly enclosed by an inner porous and an outer solid cuticle. Thus, the aculeus tip is rather solid and hence resistant to mechanical damage—a feature that was already pointed out in early descriptions ("*fort dur*"; Maupertuis 1733). On the other hand it was also claimed that this hardness makes the stinger brittle and that the very tip can easily break off (Joyeux-Laffuie 1883). However, our own observations on hundreds of scorpions showed only a few instances of broken tips and we therefore conclude that the aculeus tip is not that vulnerable. An increased hardness of certain cuticular parts in arthropods is often achieved by



Figures 9–13.—9, Close-up of one venom opening (*E. flavicaudis*), three sunken sensory hairs and many small cuticular pores. 10, High magnification of Fig. 9, showing a sunken sensory hair with a folded joint membrane at its base. 11, Relatively large pores with a split duct in the center most likely represent openings of dermal glands. 12, Relatively short sensory hairs projecting from a distinct socket occur on the proximal aculeus but not in the distal tip region. 13, Several slit sense organs are found on the stinger's surface, lying perpendicular to the long axis of the telson.

an accumulation of heavy metals (zinc, manganese, iron); for example, in insect mandibles. This has also been demonstrated in scorpion mouthparts, tarsal claws and the stinger (Schofield 2001, 2005). Since it is known that zinc incorporation into ant mandibles increases their hardness about three-fold (Schofield et al. 2003), it is very likely that the high zinc or manganese concentration in the scorpion's aculeus will also render it more resistant to wear and tear. Interestingly, this distal region of heavy metal accumulation is exactly the same region that is non-fluorescent under UV illumination, yet so far, we do not know whether there is any causal relationship between those two phenomena.

It is noteworthy that the venom openings are located subterminally; that is, not at the very tip as in a pipette, but on both sides of the aculeus (almost 100 µm from the tip). This arrangement is well known from other injection devices, such as the cheliceral fangs of spiders, the venom teeth of vipers or in hypodermic needles (Foelix 2011). From a technical viewpoint this is a superior solution because a lateral pore opening is mechanically more stable and cannot be clogged by tissue when pushed into the prey.

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